

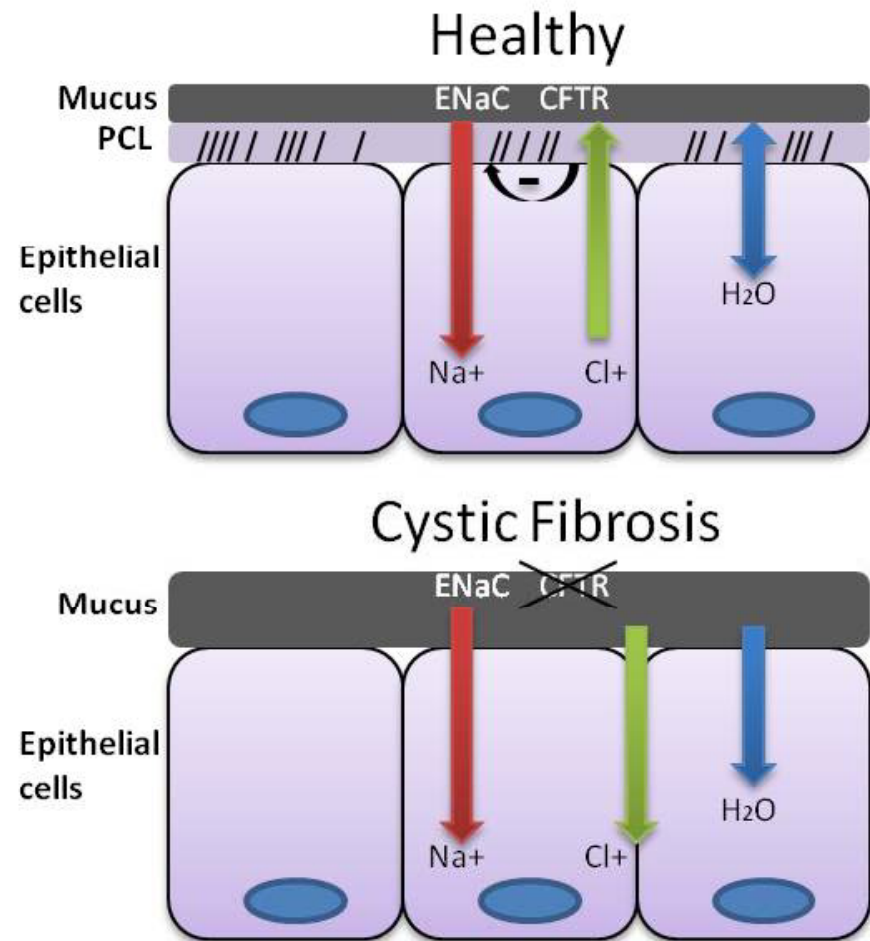
# Characterization of *Pseudomonas aeruginosa* from cystic fibrosis lung infections

Introduction to diagnostic exercise

By Trine Markussen

# Introduction

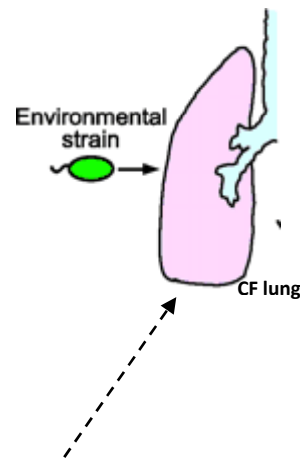
- Cystic fibrosis
- Autosomal recessive disorder
- Caused by mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene



Buchanan 2009

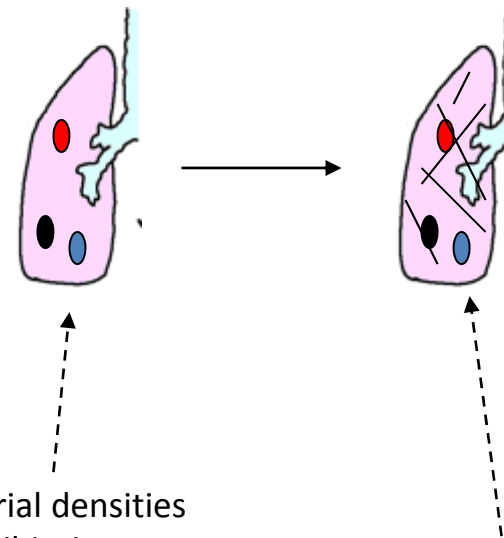
## Model of *P. aeruginosa* infections in CF patients

### Intermittent colonization (cycles of acute infections)



Periodic absence of *P. aeruginosa* in sputum, and no measurable antibodies in blood  
Wild-type strains, relative susceptible to antibiotics

### Chronic infection (decades)



High bacterial densities despite antibiotic treatment and actions of the immune system  
Diversification

Airway inflammation and progressive lung damage  
Selection

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● → ●●● : Diversification: *P. aeruginosa* undergoes substantial genetic change during chronic infections

## **The Copenhagen *P. aeruginosa* strain collection**

*P. aeruginosa* strains from CF patients have been collected since '73 by Niels Høiby

This unique strain collection represents more than 30 years of 'infection history' from a large number of patients

## Infection dynamics in CF patients: A paleontological journey

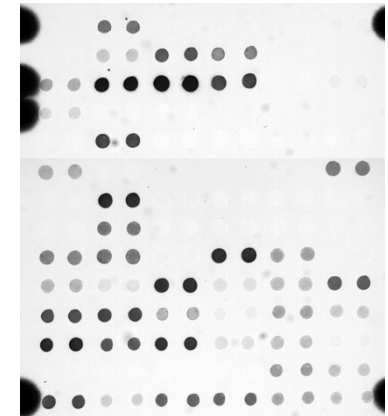
A paleontologist carefully chips rock from a column of dinosaur vertebrae



Genotyping of sequential isolates from different patients



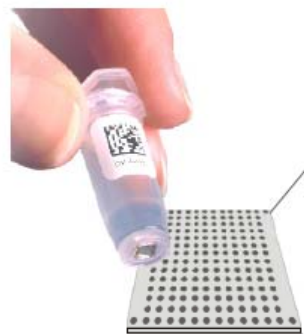
A section of the 'frozen fossil collection' of bacteria isolated from CF patients



SNP based  
genotyping

**Paleontology** is the study of the history of life based on fossil records

## DNA-chip based genotyping of *P. aeruginosa*



*P. aeruginosa* array of 14 x 14 features

M	C-45	C-46_1	C-47-1_2	SG17M-1	SG17M-8	C-specific-1	M
	C-isld.spec.1	C-isld.spec.4	C-isld.spec.5	C-isld.spec.8			
M	pKL-1	pKL-3	TB-C47-3	TB-C47-3_2	PAPI-1_pili-chap	PAPI-1_lum_bin_pro	
M	pKLC102-unknown	pKLC102-adhesin	pKLC102-metab				
	PyovRec-I	PyovRec-IIa	PyovRec-IIb	PyovRec-IIc	PyovRec-Fpv_B	LES	
	PA0636	PA0722			PAGI-1-1	PA0980	
	PA0728	PA2185	fliA-isld-1	fliA-isld-2_orfA	tRNA-Pro-isld.	PAPI-2-ac.tr.	
	PA2221	PA3835	fliA-isld-2_orfI	fliA-isld-2_orfJ	tRNA-Pro-isld.	PAPI-2-XF1753	
	ampC_7 wt	ampC_7 mut	fliC a	fliC b	exoS_1_1	exoU/PAPI-2	
	ampC_4 wt	ampC_4 mut	ampC_5 wt	ampC_5 mut	ampC_6 wt	ampC_6 mut	
	oprI b wt	oprI b mut	ampC_1 wt	ampC_1 mut	ampC_3 wt	ampC_3 mut	
	citS-1 wt	citS-1mut	citS-2 wt	citS-2 mut	oprI a wt	oprI a mut	
	fliC 1 wt	fliC 1 mut	fliC 2 wt	fliC 2 mut	alkB2 wt	alkB2 mut	
M	oriC wt	oriC mut	oprL a wt	oprL a mut	oprL b wt	oprL b mut	M

Outline of the Clondiag® chip

### Gene islands:

Fla- island (Lory), PAPI-1, -2 and -3, pKLC102, 47D7-island, PAPI-1, PAPI-2

### Variable genes & pathogenicity factors:

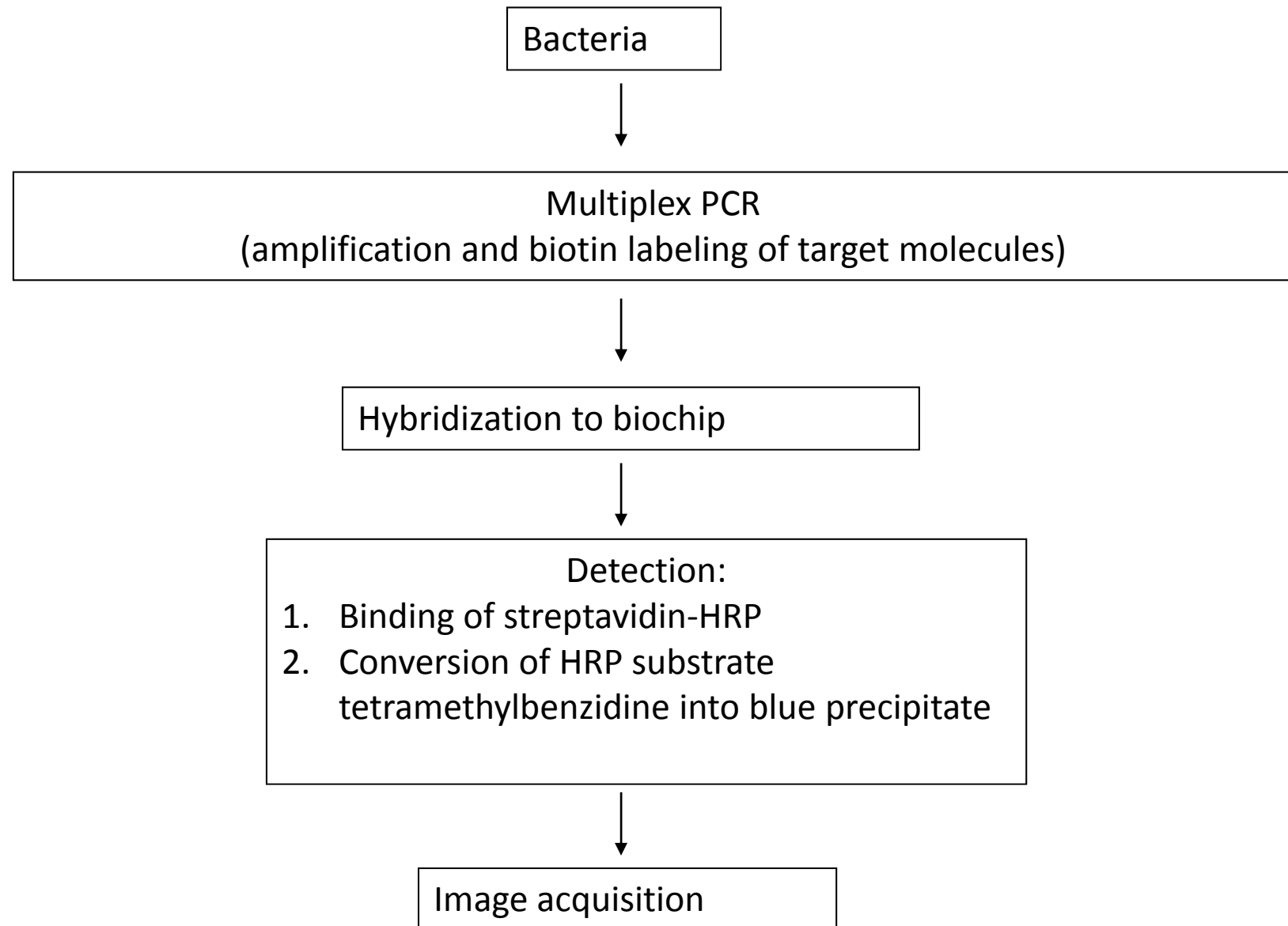
PA0636, PA0722, PA0728, PA0980, PA0987, PA2221, PA2387, PA3065, PA3291, PA3835, PA4101, *exoS*, *exoU*, *fliCa*, *fliCb*

### SNPs:

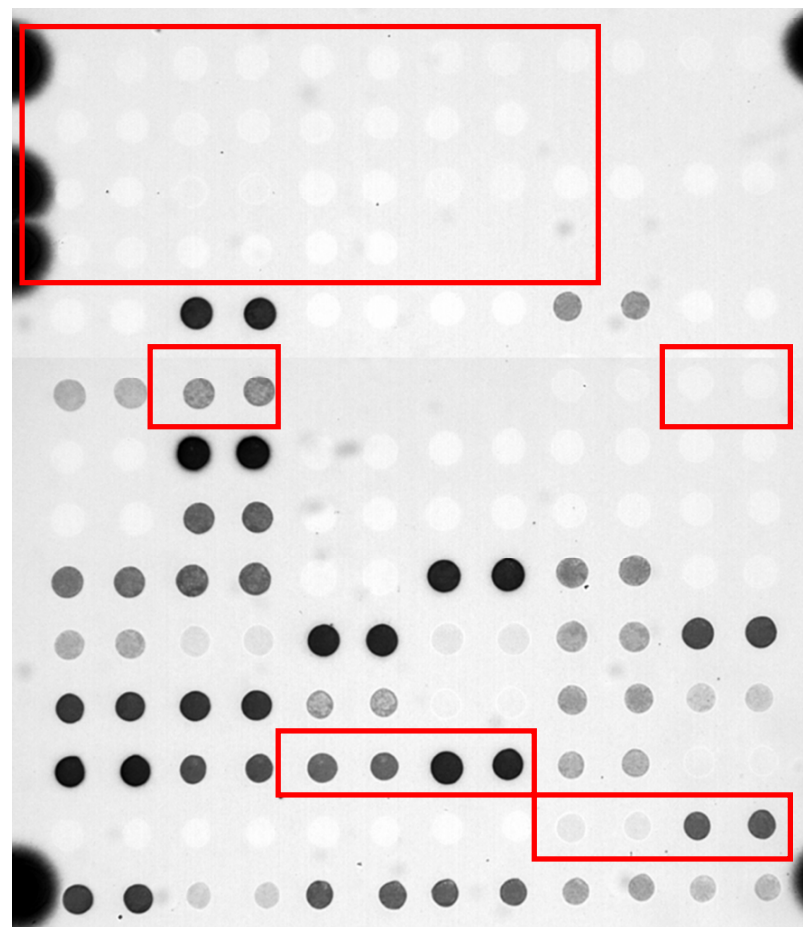
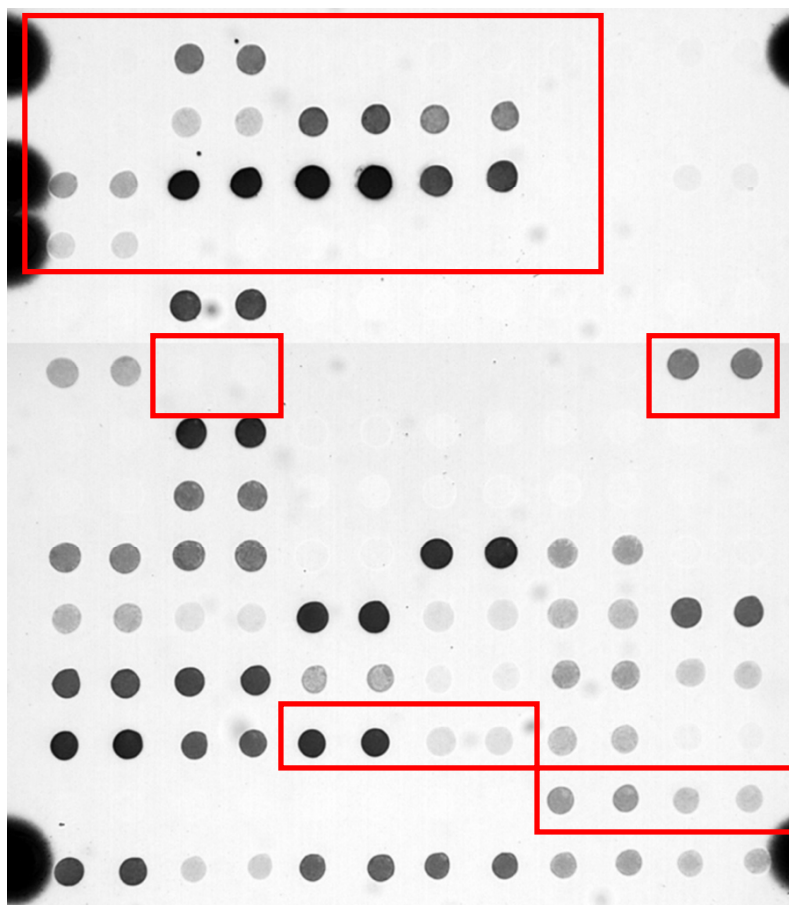
*oriC*, 2x *citS*, 6x *ampC*, *oprI*, *fliC*, *oprL*, *alkB2*

Unique sequences from SNPs (single nucleotide polymorphisms) of highly conserved genes.

## Protocol

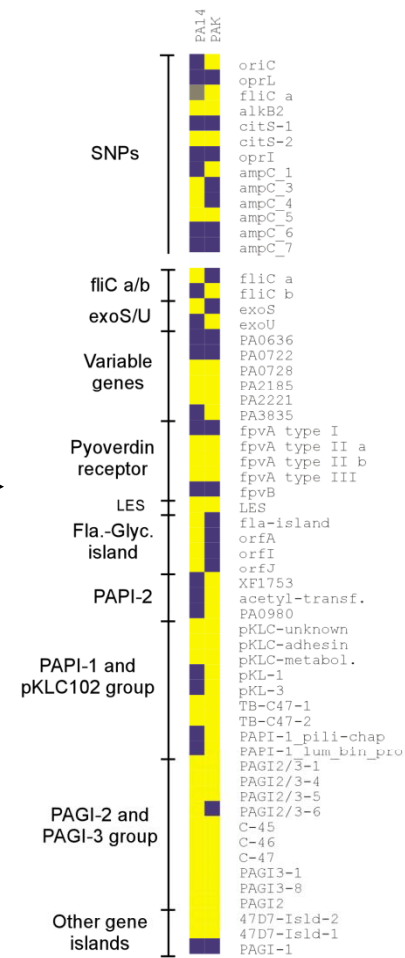
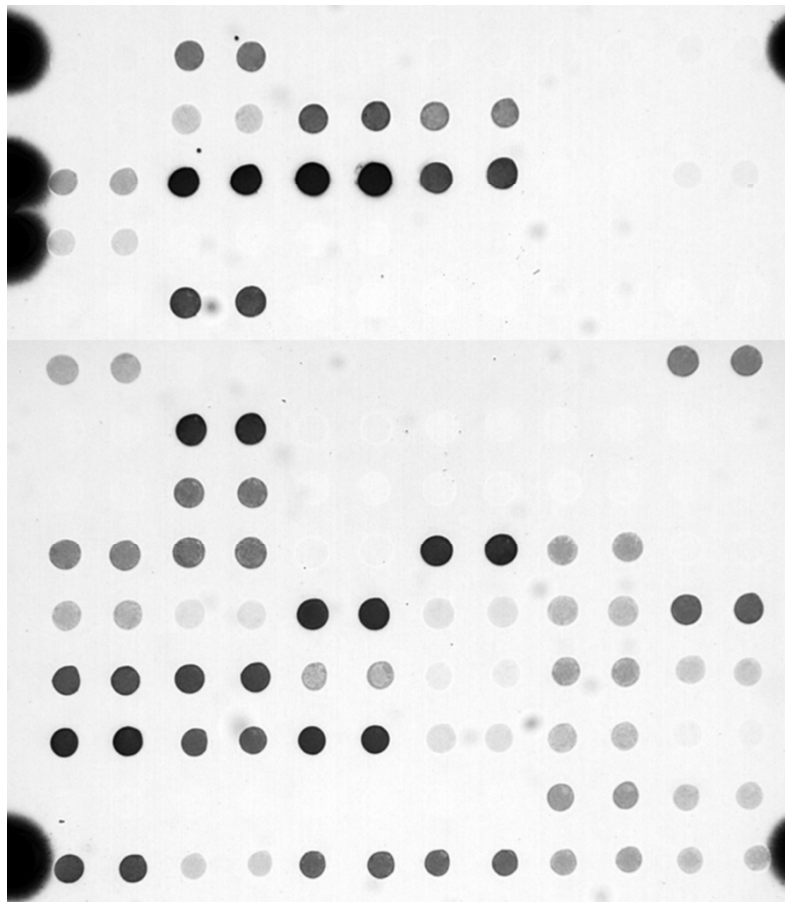


Example: 2 different clones...





# Data visualization



'barcode'

'Data  
compression'

# Infection dynamics in long-term chronically infected CF patients

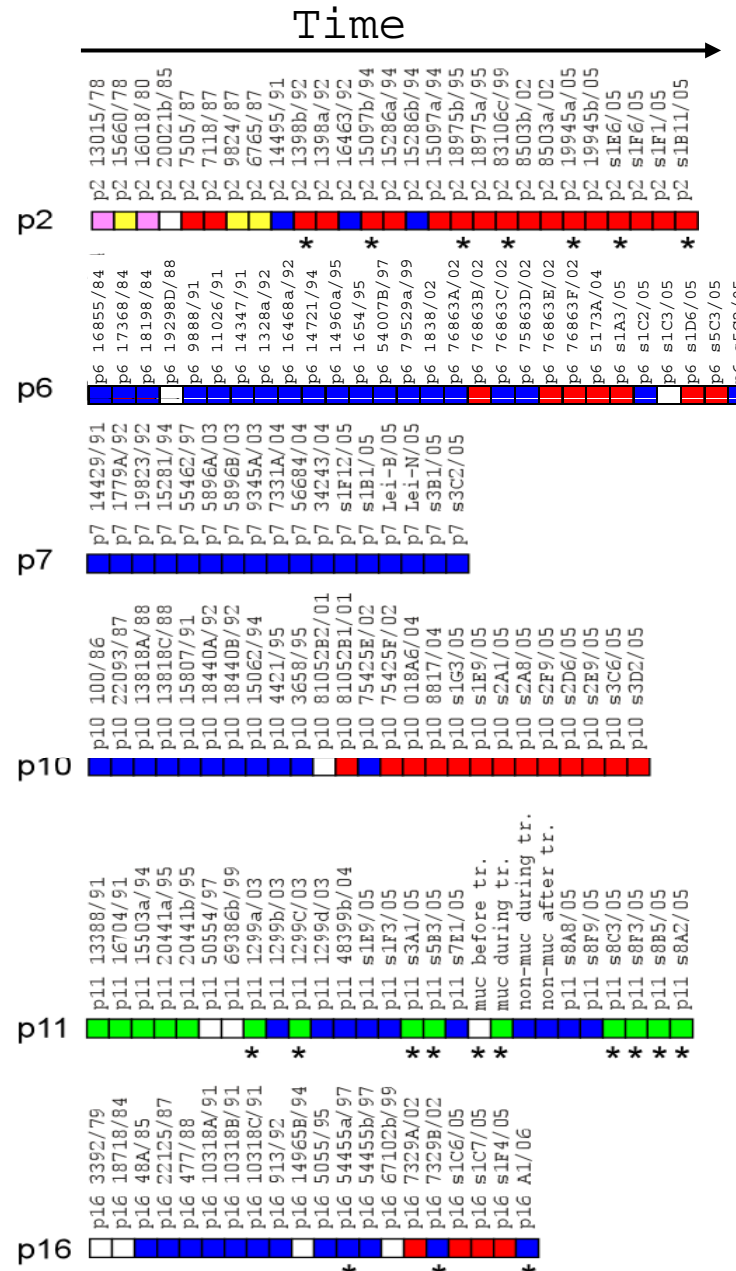
## Genotype key:

Each **Color** indicate specific genotypes

**No color** indicate unique genotypes found only once

## Phenotype key:

\* = mucoid



## Conclusions:

2 dominant clones: 'red' and 'blue'

6/6 random patients have become infected with one or both

Most long-term chronic infected patients carry 'red' and/or 'blue': We have identified the clones in >30 other patients.

*Jelsbak et al., 2007*

# The exercise

- 2 isolates
- Genotyping
  - AT-chip
- Phenotyping
  - Antibiotic resistance

## CF224

1	1987
2	1988
* 3	1992
4	1994
5	1995
6	1995
* 7	1997
8	2002
* 9	2002
10	2003